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Observations of Pituitary Hormone Injections and Ripening of Fish

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OBSERVATIONS ON PITUITARY HORMONE INJECTIONS AND RIPENING OF FISH

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D. K. Kaushik

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INTRODUCTION

A dependable source of quality fish spawn is a fundamental prerequisite for fish culture development. This is especially important inasmuch as most of the cultivable species do not breed in confined waters. Also, sport fisheries are gaining greater popularity, and subsequently the fish supply is being taxed. Still another need for fish spawn is in the ever increasing demand for bait minnows. Also, the construction of more and more dams has resulted in insurmountable obstacles for ascending and descending fish, which may ultimately result in complete destruction of some fisheries. Thus some measure of artificial propagation will have to be taken to safeguard our valuable fishery resources.

A partial solution to this problem of supplementing natural propagation is that of inducing the fish to spawn artificially in the hatchery. A method of doing this is by stimulating fish to breed by the use of pituitary hormones. These pituitary hormone-containing glands are often collected under a variety of field conditions which may involve considerable effort, time, and money. Therefore, it was my objective in this study to develop a practical refined assay on hormones using as small an amount as possible of the crude extract of pituitary suspension, and to make it simple enough that every lay fisheries man will be able to apply it, thus meeting his demand for quality fish eggs in his own hatchery when he needs it most.

REVIEW OF LITERATURE

Fish have been kept in captivity since ancient times, as far back as 2000 B.C. There are food fish, many in the Eastern countries, that have regularly been raised in ponds for decades, but they have not reproduced in captivity. One approach to induce these food fish to spawn in confined waters is suggested by the experiments of B. A. Houssay of Argentina who in 1930 injected small viviparous fish with pituitary glands extracted from another species of fish. This brought about premature birth of the young (Pickford and Atz, 1957).

For years, the chief problem in Brazillian fish culture had been to get mature freshwater food fish to spawn in captivity. Even when caught in a nearly ripe condition these species rarely completed maturation. After 2 to 3 years of experimentation, Von Ihering and Wright (1935) developed a successful technique for inducing pond fish to spawn by injecting them with pituitaries of other fish.

The most extensive use of pituitary treatment in pisciculture has been in the Soviet Union. The Russians attempted to induce captive sturgeon to spawn in 1932 by means of mammalian hormones. It was not until 1937 that N. L. Gerbil'skii was able to obtain ripe eggs and sperm from a significant number of sturgeon, Acipenser stellatus Linnaeus, that had been intracranially injected with one or two pituitaries of the same species (Pickford and Atz, 1957). Gerbil'skii's method of injecting the pituitary material inside the skull is no longer employed, probably because

of the high degree of skill required to avoid serious injury to the fish (Pickford and Atz, 1957).

In the United States, the earliest use of pituitary treatment was carried out by Hubbs (1933). The first attempt to solve piscicultural problems by this method appears to have been made by Hasler, Meyer, and Field (1940). They were able to cause female muskellunge, Esox masquinongy Mitchill, which were prevented by a dam from reaching their spawning grounds, to yield eggs for artificial incubation. Palmer et al. (1954) showed that administration of blueback salmon pituitary extract hastened the appearance of spawning coloration and the fish spawned earlier, but the fertility of the eggs was lowered. Ramaswamy, Hasler, and Meyer (1955), using carp pituitary, produced tubercles in the male minnow but not in the female. Ramaswamy and Sunderaj (1956) indicated that administration of pituitary glands induced spawning in catfish during the breeding season.

MATERIALS AND METHODS

Test animals

Goldfish, Carassius auratus Linnaeus, were selected as the experimental fish, while carp, Cyprinus carpio Linnaeus; brown trout, Salmo trutta Linnaeus; mountain whitefish, Prosopium williamsoni Girard; Bonneville cisco, Prosopium gemmiferum Snyder; largemouth bass, Micropterus salmoides Lacepede; and goldfish were used as donors of pituitary glands.

Goldfish were used as the recipient because they are adaptable to laboratory conditions. In some experiments only male goldfish were used as the recipient as it was observed that they required a much lower dose for a given response and that they exhibited a more clear-cut and earlier strippability, which in turn served as a good end point for assaying different pituitary preparations.

Removal of pituitary glands and collection

A diagonal cut was made on the skull from just above the eye to very near the vertebral column, and the bone was removed. The pituitary gland is embedded just below the point where the infundibulum is located, in a small cavity in the floor of the skull.

Pituitaries from different species were collected in the field from Bear Lake, Logan River, Bear River Bird Refuge, and one Bodrero Pond. Collection of pituitary continued from November 1959 until late April 1960. About 215 carp, 51 brown trout, 50 mountain whitefish, 20 Bonneville cisco, and 20 largemouth bass pituitaries were collected and preserved.

Goldfish pituitaries were collected in the laboratory and preserved in the same way. Carp and largemouth bass pituitaries were collected before the breeding season in April and May 1960. Bonneville cisco pituitaries were collected about a week before its breeding time, in the middle of January 1960. Brown trout and mountain whitefish pituitaries were collected after they had spawned, in January, February, and March 1960. About 70 carp pituitaries were supplied by the Utah Fish and Game Department. These carp pituitaries were collected in July 1960 when about 50 per cent of the carp had spawned.

Preservation of pituitary glands

As soon as pituitary glands were removed in the field, they were put in absolute alcohol in dark colored, screw-capped vials. These pituitaries were brought to the laboratory, washed two or three times in alcohol, and replaced in the vials with fresh alcohol. These vials were placed in a desiccator with calcium chloride, after which they were stored in a refrigerator.

Preparation of extracts for injection

The alcohol-preserved pituitary glands were first dried on filter paper and then weighed on an analytical balance. The pituitaries were powdered in a tissue homogenizer and then diluted in distilled water in a small graduated cylinder.

Injection

Injections were given at various levels of pituitary concentrations in different experiments. Both intraperitoneal and intramuscular injections were given in order to determine differences. Intraperitoneal injections were given at the base of the pelvic fin where the skin is soft. Intramuscular injections were given in the caudal peduncle. The

injection was given by lifting a scale with the needle, piercing the muscle slightly, and then tilting the syringe parallel to the body of the fish to avoid any injury to internal parts of the fish. No two injections on one individual were given in the same spot. Injections were given according to body weight of the fish. Each fish was weighed before the injection on a torsion balance, which gives a quick and accurate reading. Injections were made with a 1 cc tuberculin syringe marked to .01 cc, which had a sharp, thin needle. A similar procedure was followed for the control fish, except that they were injected with distilled water.

Sexing of experimental fish

Efforts were made to segregate male and female goldfish by observing external characteristics. It was noticed that in most cases the body tissue of the females, just behind and toward the side of the pelvic fin, was somewhat softer than that of the males. Also, males have a narrow ventral profile, while in females the ventral profile is broad and somewhat flat. These two tests gave an accuracy of about 90 per cent.

Experimental plan

Acclimatization.--Goldfish were kept in 60-gallon aquaria for approximately a week after shipment. They were fed a small, crumbled trout food. Feeding was discontinued a day before the experiment started. This off-feed practice was to keep organic wastes to a minimum. These fish were kept in small aquaria at room temperature before finally putting them in the experimental 1-gallon bottles.

Experimental practices.--All bottles in the experiment were washed and rinsed thoroughly. These bottles were allowed to air dry before being filled with water. Logan City tap water was used in the experiment.

The water was allowed to stand in the bottles for about 6 hours before the experiment to stabilize any exchange of gases. Water in the bottles was well aerated 3 to 4 hours before the experiment.

Experimental plant.--All experiments were at room temperature (about 75° F.). One fish was kept in each of the open-mouthed bottles, which had a capacity of 3 liters of water. All bottles were well aerated throughout the experiment by plastic tubes, each terminating with a carbon stone, and connected to the aerators through a keyboard arrangement. Uniform aeration in all bottles could be maintained by adjusting the screw holders on the keyboard for each bottle (Figure 1). Some experiments were conducted in aquaria of 3-gallon capacity.

Experimental design and analysis.--The design used in all the experiments was completely randomized. The data were grouped into three classes: fully strippable, intermediate, and hard. Fully strippable fish were those which stripped easily with a slight rubbing of hands on the side of the fish. Intermediates were those from which only a few eggs or milt came with somewhat more pressure on the belly. Hard fish were those which could not be stripped even with excessive pressure. These were assumed to be not affected by the pituitary injections. For statistical analyses, however, the intermediates were grouped with the strippable fish; and thus the whole data were classified into two groups, e.g. affected and not affected. The analyses were carried out by the chi-square test. The Brandt and Snedecor formula given below was applied for all tables having more than two columns and rows.

$$x^2 = \frac{\Sigma a_i p_i - \bar{p}\,\Sigma(a_i)}{\bar{p}\bar{q}}$$

Where a_i = number affected under ith treatment

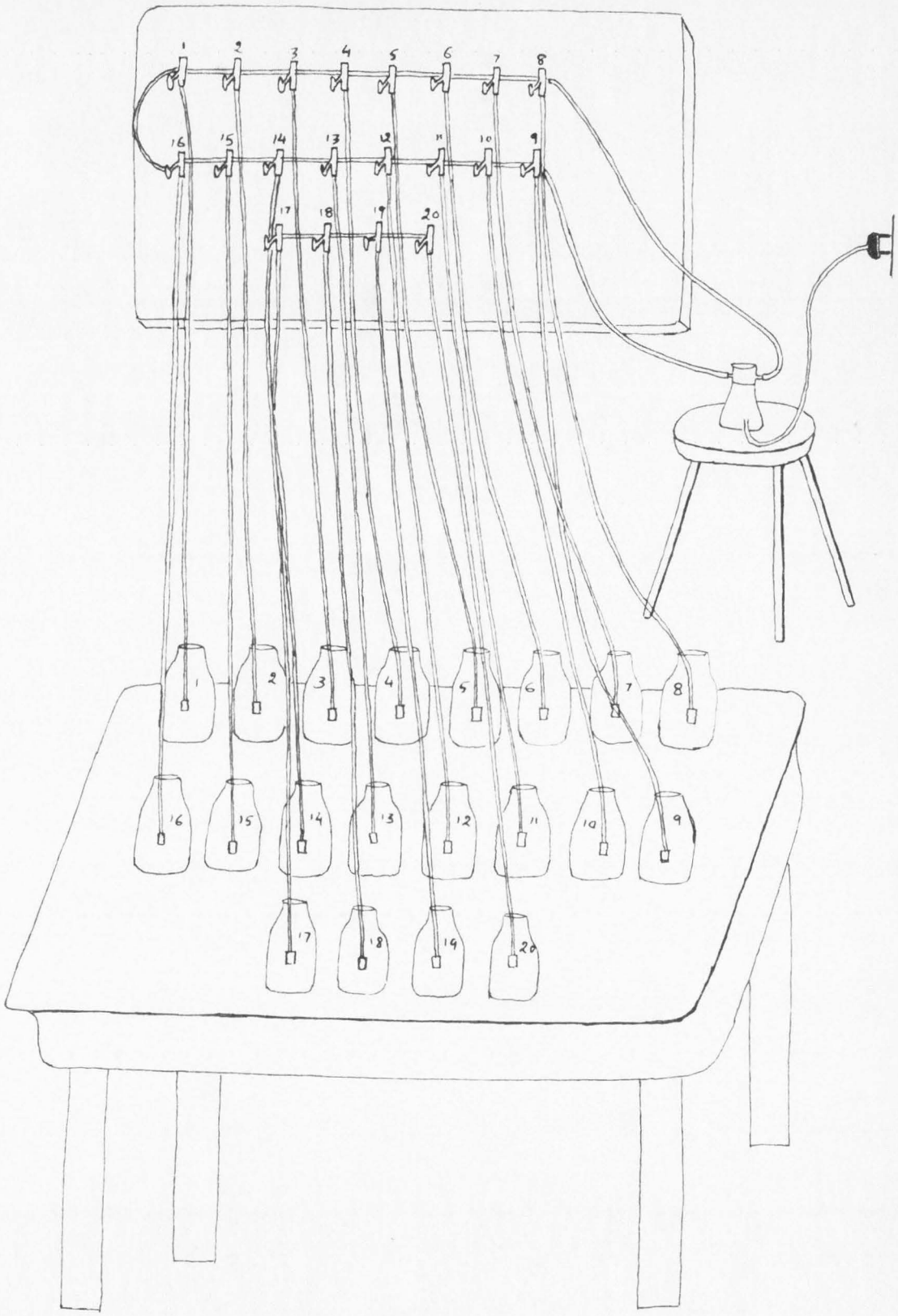

Figure 1. Experimental plant

$\bar{p}$ = overall proportion affected

$\bar{q}$ = $1-\bar{p}$

The correction of continuity was made for all 2 x 2 tables.

RESULTS AND DISCUSSION

Study on pituitary hormone injections

Effect of different levels of carp pituitary injections on male and female goldfish.--Pituitaries used in these experiments were collected in April and May of 1960, before the carp had spawned. The carp averaged about 1.5 feet in length, and pituitaries were used from both sexes.

Carp pituitaries were injected in male goldfish at 1.83[1], 0.91, 0.45, and 0.22 mg%. The fish were processed 12 hours after injection. It was observed that at 1.83 mg% all male goldfish were strippable within 12 hours. At 0.91 mg%, while half of them were fully ripe, 33.3 per cent reached the intermediate stage and 16.7 per cent remained in the hard condition. A lower dose than 0.91 mg% of carp pituitary did not affect male goldfish within this 12-hour period. The response of males at different levels of pituitary injections is given in Table 1 (illustrated in Figure 2).

It was observed in earlier experiments that female goldfish were not affected at 1.83 mg% of carp pituitary injections within 12 hours. Therefore, another experiment was set up in which doses of higher levels of carp pituitary injections were tried on female goldfish. Injections were made at 1.83 , 2.33, 3.08, and 4.16 mg% of carp pituitary from the

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[1]1.83 mg% means 1.83 mg of pituitary injected to 100 grams of fish.

Table 1. Response of male goldfish at different levels of carp pituitary injections.

Treatment	Level of injection mg%	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	1.83	100	--	--	100	--
	0.91	50	33.3	16.7	83.3	16.7
	0.45	--	--	100	--	100
	0.22	--	--	100	--	100
Control	$D.H_2O$	--	--	100	--	100

Fish were processed 12 hours after injection.

Statistical Analysis[1]

Source of Variation	d/f	X^2	Probability
Among treatments	4	11.1	.995
Within levels of injection	3	41.2	.995
Treatment vs. control	1	6.8	.99
1.83 mg% vs. 0.91 mg%	1	0.54	.10
1.83 mg% vs. rest	1	22.6	.995

[1]Statistical analysis indicates that there is a difference in the fraction of fish affected among treatments and also within levels of injection. Fraction of fish affected in the group injected with 1.83 mg% was maximum. This is significant with a probability of .995.

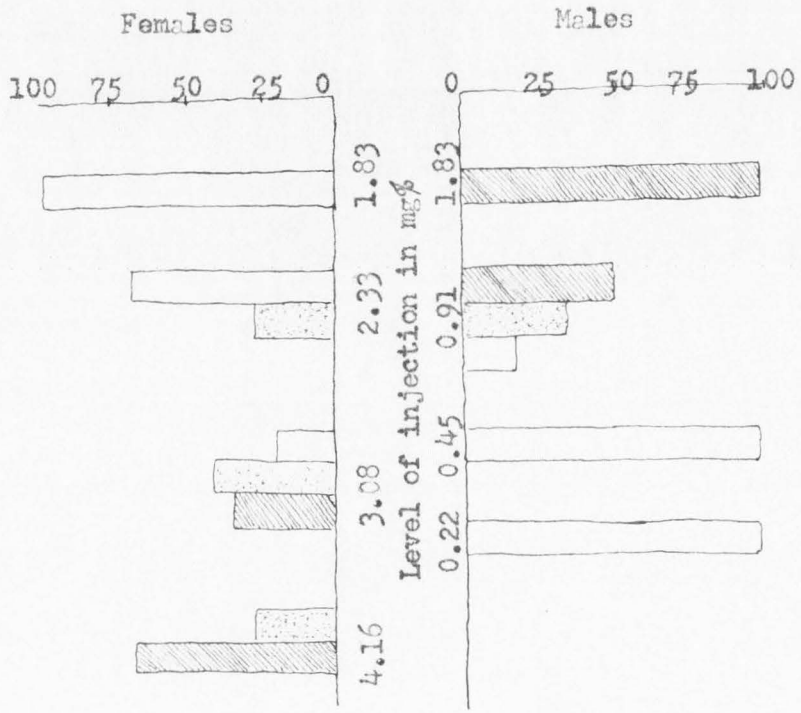


Figure 2. Response of male and female goldfish at different levels of carp pituitary injections. Fish were processed 12 hours after injection.

same stock used earlier for male goldfish. The experimental female goldfish were examined 12 hours after injection. Again at the 1.83 mg% injection of carp pituitary, no female goldfish were affected within 12 hours. At 2.33 mg%, 27.8 per cent of the female goldfish reached the intermediate stage. At 3.08 mg%, 35.3 per cent were fully strippable, 41.1 per cent were intermediate, and 23.6 per cent were not affected. However, when the level of injection was raised to 4.16 mg%, all female goldfish were affected: 72.2 per cent were fully ripe, and 27.8 per cent reached the intermediate stage. The response of female goldfish at different levels of carp pituitary injection is given in Table 2 (illustrated in Figure 2).

It was observed that male goldfish required a much lower dose of carp pituitary than females. The males reached ripeness at 1.83 mg% injection in 12 hours, while females needed a higher dose of 4.16 mg% of carp pituitary to reach ripeness within the same time.

Effect of carp pituitary injections on male and female goldfish in successive hours after injection.--In one experiment, female goldfish were injected with 4.16 mg% of carp pituitary. The carp pituitaries used were from the stock collected before their breeding period. The experimental goldfish were observed every 2 hours. As shown in Table 3 (illustrated in Figure 3), none of the female goldfish was affected in the first 4 hours after injection. However, after 6 hours, there were 10 per cent which reached the intermediate stage. After 12 hours 70 per cent of the fish were fully ripe,and 25 per cent were at the intermediate stage. There were only 5 per cent which were not affected.

In another experiment female goldfish were injected with 4.16 mg% of carp pituitary from the same stock. In this case fish were observed for 30 hours at intervals of 6 hours. After the injection, 83.3 per cent

Table 2. Response of female goldfish at different levels of carp pituitary injections.

Treatment	Level of injection mg%	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	1.83	--	--	--	--	--
	2.33	--	27.8	72.2	27.8	72.2
	3.08	35.3	41.1	23.6	76.4	23.6
	4.16	72.2	27.8	--	100	--
Control	$D.H_2O$	--	--	100	--	100

Fish were processed 12 hours after injection.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	4	60.4	.995
Within levels of injections	3	43.3	.995
Treatment vs. control	1	13.6	.995
4.16 mg% vs. rest	1	20.3	.995
4.16 mg% vs. 3.08 mg%	1	2.7	.90
4.16 mg% vs. 2.33 mg%	1	17.3	.995
3.08 mg% vs. 2.33 mg%	1	6.4	.99

[1]Analysis indicates that there is a difference in the fraction of fish affected among treatments and also within levels of injection. The fraction of fish affected in the group injected at 4.16 mg% versus the rest is significant with a probability of .995. Fraction of fish affected at this level of injection was maximum.

Table 3. Response of female goldfish at successive hours after carp pituitary injection of 4.16 mg%.

Treatment	Response in percentage					
	After 2 hours	4 hours	6 hours	8 hours	10 hours	12 hours
Treated fish	-	-	10a	10a 10b	35a 30b	70a 25b
Control ($D.H_2O$)	-	-	-	-	-	-

- Hard
a Intermediate
b Strippable

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	4	42.5	.995
Within hours	3	37.8	.995
Treatment vs. control	1	14.9	.995

[1]Analysis provides strong evidence that a difference in the fraction of fish affected exists between treated and control fish. Also, there is a difference in the fraction of fish affected at successive hours after the injection. As indicated above they are all significant with a probability of .995.

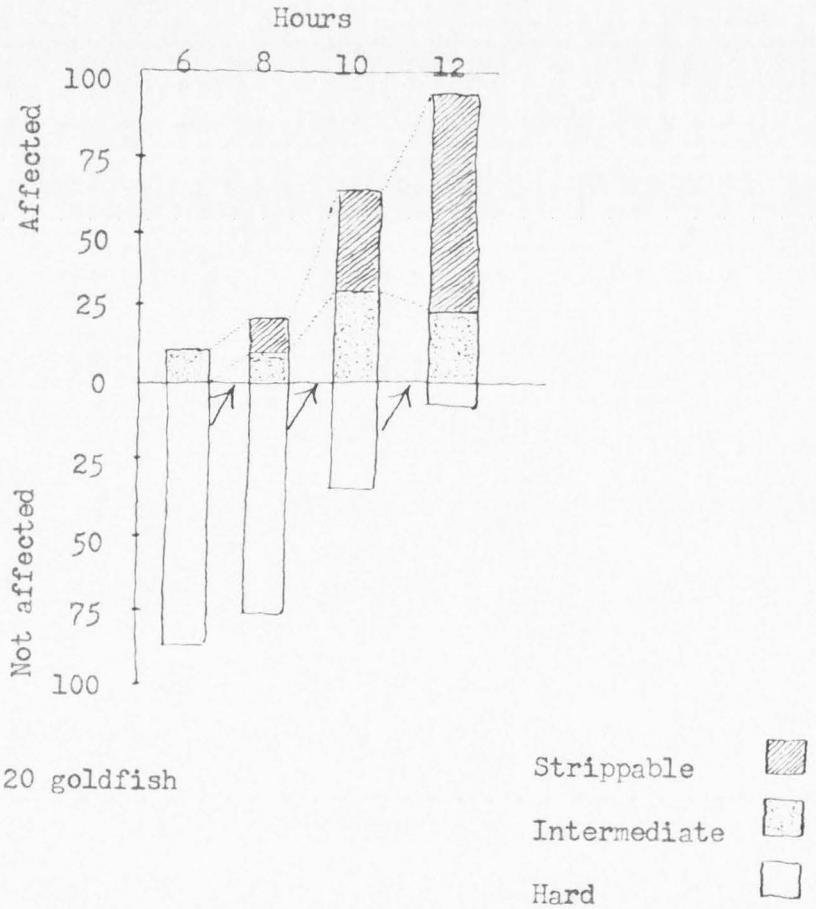


Figure 3. Response of female goldfish at successive hours after the carp pituitary injection of 4.16 mg%.

of the females were affected within 12 hours, and by 18 hours all were affected. After 30 hours, there was an 8 per cent decline in strippable fish. This difference, however, is not significantly different from those processed at 18 and 24 hours (Table 4).

In the third experiment both male and female goldfish were injected with 1.83 mg% of carp pituitary. Pituitaries used were from the same stock. The fish were observed after every 6 hours (Table 5). As already noted in earlier experiments, all males were strippable after 12 hours at this level. Females, which were not affected at this level within 12 hours in previous experiments, started to show signs of the effect of the injection after 24 hours. The intermediate stage had been reached by 11.1 per cent after 24 hours. After 30 hours, the intermediates represented 37.5 per cent. The response of male and female goldfish, when treated with 1.83 and 4.16 mg% of carp pituitary respectively, is illustrated in Figure 4.

Comparison of effect on male and female goldfish of one massive dose of carp pituitary and the same amount in a series of small doses.--In this experiment one group of goldfish (including males and females) were given carp pituitary injection at 1.83 mg%. The other group of goldfish were given carp pituitary injections in a series of small doses: 0.91 mg% at the initial hour followed by 0.45 mg% after 3 hours and 0.45 mg% after another 2 hours. Fish were observed after every 2 hours.

When given injections in a series of doses, 68.7 per cent of the males reached ripeness after 6 hours as compared to only 25 percent of the males which reached this stage when given injections in one massive dose (Table 6 and Figure 5). After 8 hours, all males were strippable in the group of fish injected with a series of doses as compared to 81.2 per

Table 4. Response of female goldfish at successive hours after carp pituitary injection of 4.16 mg%.

Treatment	Time interval in hours	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	6	25	8.3	66.7	33.3	66.7
	12	66.6	16.7	16.7	83.3	16.7
	18	75	25	--	100	--
	24	91.7	8.3	--	100	--
	30	83.3	16.7	--	100	--
Control (D. H_2O)	30	--	--	100	--	100

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	5	51.5	.995
Within time intervals	4	27.0	.995
Treatment vs. control	1	28.9	.995
12 hours vs. rest	1	.64	.10

[1]There is a significant difference in the fraction of affected fish among the treatments, within time intervals at successive hours, and also treatment versus control fish. They all are significant with a probability of .995. There is a gradual increase in the fraction of fish affected with time. By 18 hours after injection all fish were affected.

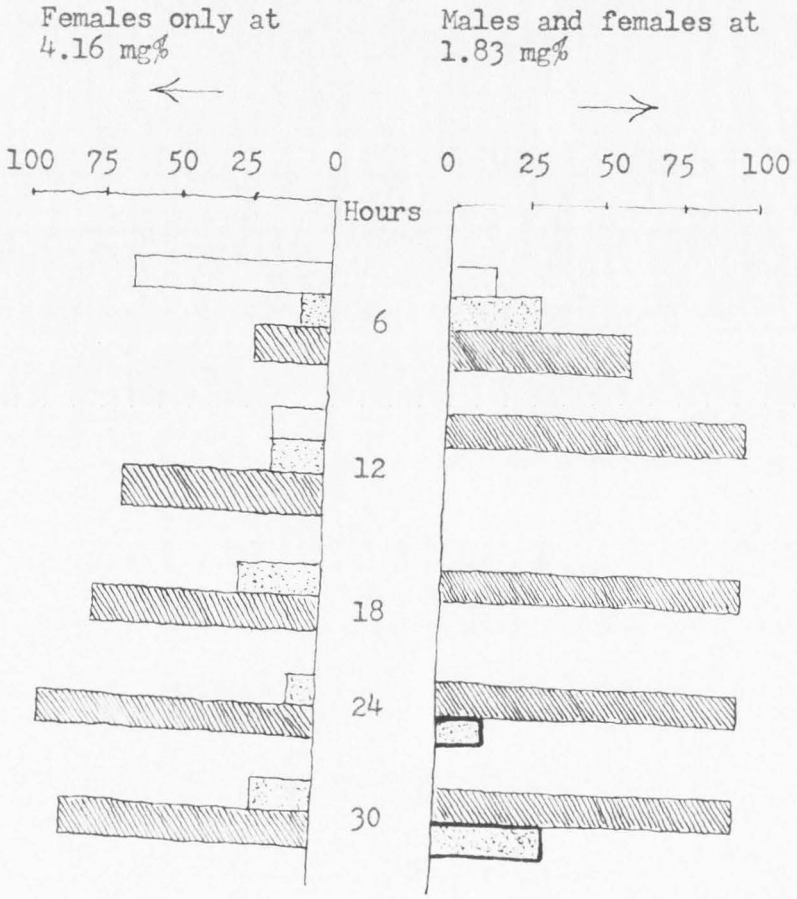
Table 5. Response of male goldfish at successive hours after carp pituitary injection of 1.83 mg%.

Treatment	Time interval in hours	Sex	Response in percentage: Strippable	Intermediate	Hard	Total percentage: Affected	Not affected
	6	♀	--	--	100	--	100
		♂	57.1	28.6	14.3	85.7	14.3
	12	♀	--	--	100	--	100
		♂	100	--	--	100	--
	18	♀	--	--	100	--	100
		♂	100	--	--	100	--
	24	♀	--	11.1	88.9	11.1	88.9
		♂	100	--	--	100	--
	30	♀	--	37.5	62.5	37.5	62.5
		♂	100	--	--	100	--
Control $D.H_2O$	30	♀	--	--	100	--	100
		♂	--	--	100	--	100

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	5	22.3	.995
Within males	5	4.5	.10
Within females	5	14.6	.975
Treatment vs. control	1	13.4	.995

[1]There is a slight difference in the fraction of males affected at successive hours. However, females are significantly different with a probability of .975 at successive hours.



Sample size: 50 females for the 4.16 mg%
48 females for the 1.83 mg%
42 males for the 1.83 mg%

Figure 4. Response of male and female goldfish at successive hours after carp pituitary injections of 1.83 and 4.16 mg% respectively. Some females were also given injection along with males at 1.83 mg%.

Table 6. Response of male and female goldfish after one massive dose of carp pituitary at 1.83 mg% and the same amount given by a series of small doses: 0.91 mg% at the initial hours, 0.45 mg% after 3 hours, and again 0.45 mg% after another 2 hours.

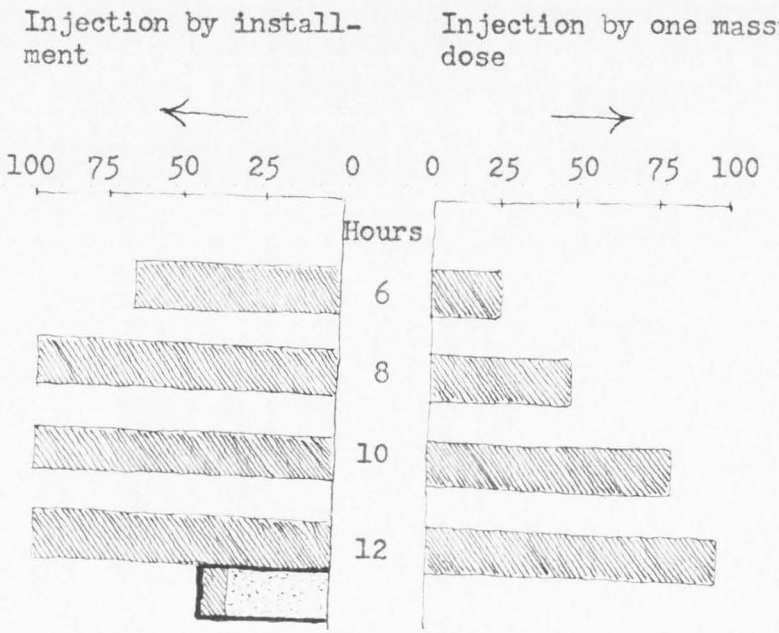
Treatment	Dose of injection	Sex	Response in percentage After 2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs.	12 hrs.
Treated fish	One massive dose	♀	-	-	-	-	-	-
		♂	-	-	25b	50b	81.2b	100b
	By a series of small doses	♀	-	-	-	-	-	35.7a 7.1b
		♂	-	-	68.7b	100b	100b	100b
Control $D.H_2O$	One massive dose	♀	-	-	-	-	-	-
		♂	-	-	-	-	-	-
	By a series of small doses	♀	-	-	-	-	-	-
		♂	-	-	-	-	-	-

- \- Hard
- a Intermediate
- b Strippable

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	3	16.2	.995
Treatment vs. control	1	11.4	.995
One massive dose vs. series of small doses	1	3.5	.90
Within males (one massive dose)	3	23.0	.995
Within males (by a series of small doses	3	16.3	.995
One massive dose (6 hours) vs. series of small doses	1	8.03	.995

[1]The analysis indicates a difference in the fraction of fish affected by injections in one massive dose and those receiving a series of doses. After 6 hours the difference in the fraction of fish affected in these two types of injections is significant with a probability of .995.



Sample size: 30 goldfish for injection by one massive dose (14♀ and 16 ♂)

30 goldfish for injection by a series of small doses (14♀ and 16♂)

Males
Females
Strippable
Intermediate

Figure 5. Response of male and female goldfish after one massive dose of carp pituitary at 1.83 mg% and giving the same amount by a series of small doses: 0.91 mg% at the initial hour, 0.45 mg% after 3 hours, and again 0.45 mg% after another 2 hours.

cent of the males which reached strippable stage in the group of fish that were injected in one massive dose. Females also exhibited better response when they were given a series of injections (Figure 5).

Effect of male and female goldfish pituitaries on goldfish.-- Pituitaries of both sexes of goldfish were collected in the laboratory. Due to a shortage of pituitaries, they were from treated fish used in other experiments. The male goldfish pituitaries included those from the strippable fish, the intermediates, the hard, and the control fish. Similarly, the female goldfish pituitaries were collected from the same sources. The fish used for the experiment were, however, from fresh stock.

In one of the experiments, male goldfish received male and female goldfish pituitaries at 0.45 mg%. The fish were processed after 24 hours. Female goldfish pituitary injected into male goldfish was more effective than male goldfish pituitary to males (Table 7, Figure 6). Female goldfish pituitary injected into males was about 30 per cent better in total effect (strippable and intermediates included) than male goldfish pituitary to males. There was a difference of about 50 per cent among the strippable fish.

In another experiment male and female goldfish pituitaries from the same stock were administered to female goldfish at 4.16 mg%. The fish were processed after 24 hours (Table 8). Here, too, the female goldfish pituitary injected to female goldfish was superior in its effect to male goldfish pituitary to female goldfish (Table 8, Figure 7). Butler (1940) indicated female pituitaries are more effective than male in stimulating the gonads of young goldfish. Palmer (1954) and others found no antagonistic action in male or female sockeye salmon,

Table 7. Response of male goldfish when treated with male and female goldfish pituitary at 0.45 mg%.

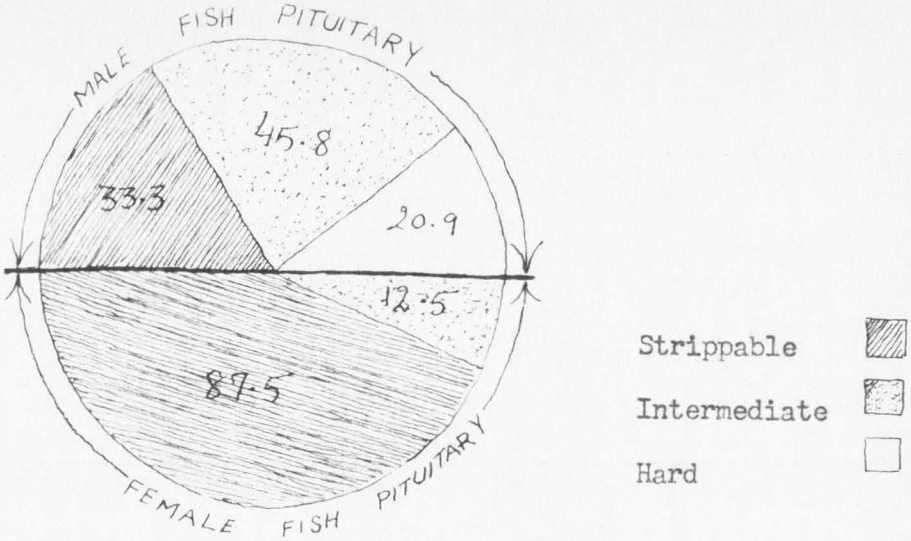
Treatment	Source of pituitary	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	♂ goldfish	33.3	45.8	20.9	79.1	20.9
	♀ goldfish	87.5	12.5	--	100	--
Control $D.H_2O$		--	--	100	--	100

Fish were processed 24 hours after treatment.

Statistical Analysis[1]

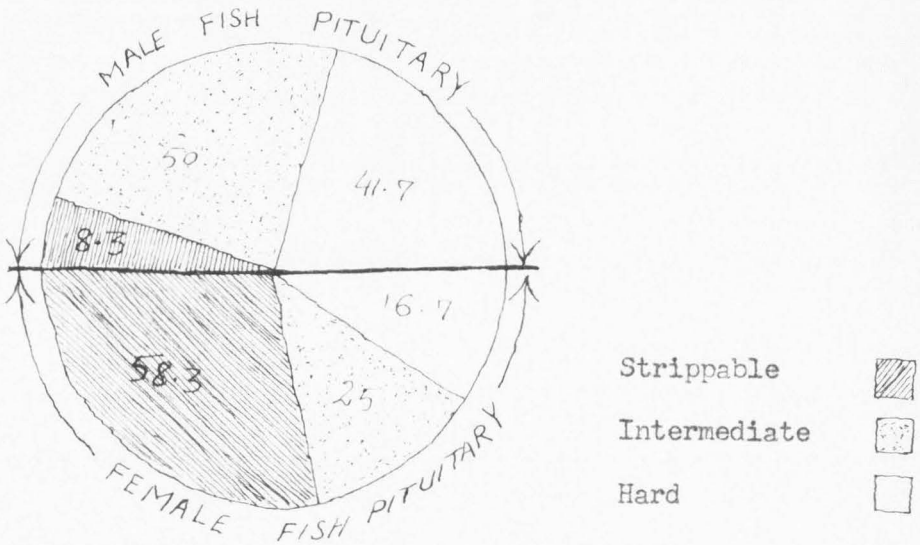
Source of variation	d/f	X^2	Probability
Among treatments	2	48.5	.995
Treatment vs. control	1	42.4	.995
♂ goldfish pituitary vs. ♀ goldfish pituitary	1	8.03	.995

[1]Male and female goldfish pituitary injections to goldfish are significantly different with a probability of .995.



Sample size: 48 male goldfish, 24 for each treatment

Figure 6. Response (in percentage) of male goldfish when treated with male and female goldfish pituitaries at 0.45 mg%. Fish were processed 24 hours after injection.



Sample size: 24 female goldfish, 12 for each treatment

Figure 7. Response (in percentage) of female goldfish when treated with male and female goldfish pituitaries at 4.16 mg%. Fish were processed 24 hours after injection.

Table 8. Response of female goldfish when treated with male and female goldfish pituitary at 4.16 mg%.

Source of pituitary	Response in percentage			Total percentage	
	Strippable	Intermediate	Hard	Affected	Not affected
♂ goldfish	8.3	50	41.7	58.3	41.7
♀ goldfish	58.3	25	16.7	83.3	16.7

Fish were processed 24 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
♂ pituitary vs. ♀ pituitary	1	3.2	.90

[1]There is a significant difference in the fraction of fish affected when treated with male and female goldfish pituitary (probability .90).

Oncorhynchus nerka Walbaum, with pituitary substance of the opposite sex. On the other hand, Ball and Bacon (1954) declared that pituitaries from male carp were not as effective on female creek chub, Semotilus atromaculatus Mitchill, as those from females, while glands from female carp were equally effective on both sexes of creek chub.

Effect of mature and immature goldfish pituitary injections on goldfish.--Mature and immature goldfish pituitaries were collected in the laboratory from female goldfish only. These females were selected from fresh stock. Pituitaries dissected from females were considered as "mature" if the volume of the gonads was large and the size of the eggs indicated full development. "Immature" pituitaries were designated as those which came from fish with underdeveloped gonads. Pituitaries from treated fish were not included in this collection.

In one of the experiments, these mature and immature goldfish pituitaries were injected into two groups of male goldfish, both at 0.45 mg%. Fish were processed 24 hours after the injection. It was observed that 76 per cent of the fish were strippable among the males when given injections from mature goldfish pituitary as compared to only 11.5 per cent of the fish that were given injections from immature fish pituitary (Table 9, Figure 8).

In another experiment, mature and immature goldfish pituitaries were injected into female goldfish at the 4.16 mg% level. The fish were processed 16 hours after injection. Here again mature goldfish pituitary was found to be more effective than immature goldfish pituitary (Table 10, Figure 9).

Effect of injected and uninjected goldfish pituitary injections to goldfish.--Injected goldfish pituitaries were collected in the laboratory

Table 9. Response of male goldfish when treated with mature and immature goldfish pituitary at 0.45 mg%.

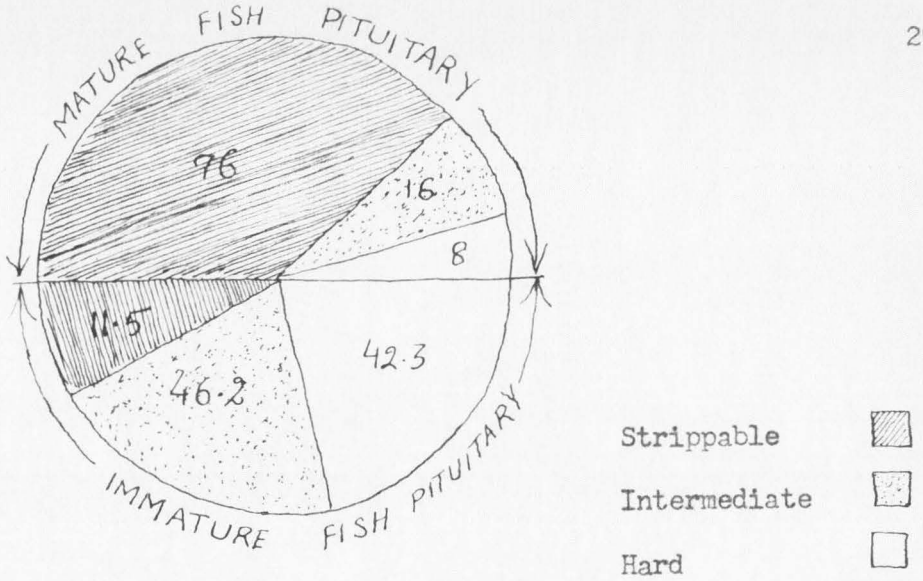
Treatment	Source of pituitary	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	Mature fish	76	16	8	92	8
	Immature fish	11.5	46.2	42.3	57.7	42.3
Control	$D.H_2O$	--	--	100	--	100

Fish were processed 24 hours after injection.

Statistical Analysis[1]

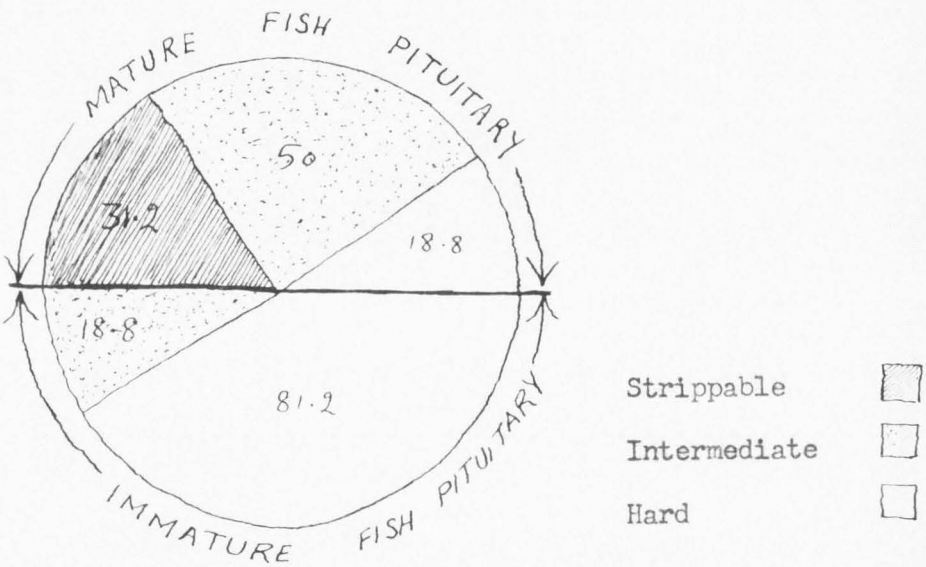
Source of variation	d/f	X^2	Probability
Among treatments	2	7.9	.975
Treatment vs. control	1	23.3	.995
Mature fish pituitary vs. immature fish pituitary	1	6.1	.975

[1]The fraction of male fish affected when injected with mature and immature fish pituitary is significantly different with a probability of .975.



Sample size: 50 male goldfish, 25 for each treatment

Figure 8. Response in percentage of male goldfish when treated with mature and immature goldfish pituitaries at 0.45 mg%. Fish were processed 24 hours after the injection.



Sample size: 32 female goldfish, 16 for each treatment

Figure 9. Response in percentage of female goldfish when treated with mature and immature goldfish pituitaries at 4.16 mg%. Fish were processed 24 hours after injection.

Table 10. Response of female goldfish, when treated with mature and immature goldfish pituitary at 4.16 mg%.

Treatment	Source of pituitary	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	Mature fish	31.2	50	18.8	81.2	18.8
	Immature fish	--	18.8	81.2	18.8	81.2
Control	$D.H_2O$	--	--	100	--	100

Fish were processed 16 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	2	19.6	.995
Treatment vs. control	1	4.7	.95
Mature fish pituitary vs. immature fish pituitary	1	10.1	.995

[1]The fraction of female goldfish affected when injected with mature and immature fish pituitary is significantly different with a probability of .995.

from treated female goldfish used in other experiments. These pituitaries were collected from only those treated females which were fully strippable and which had well developed gonads. Pituitaries from intermediates or hard fish were not included. Uninjected fish pituitaries were collected from untreated female goldfish which were in hard condition but which had fully developed gonads. In this study only male goldfish were used as experimental fish. The injected and uninjected goldfish pituitaries thus collected were injected into two groups of male goldfish each at 1.83 mg%. The fish were examined 16 hours after injection.

Some 62.5 per cent of the males administered with uninjected goldfish pituitary were ripe 16 hours after the injection as compared to only 18.2 per cent of the males treated with injected goldfish pituitary (Table 11 and Figure 10).

Effect of pituitaries from different donors on goldfish.--The effects of pituitary injections from Bonneville cisco, brown trout, mountain whitefish, largemouth bass, and goldfish on male goldfish were studied. Goldfish were injected with 1.83 mg% of the pituitary from the donor. The fish were examined 12 hours after treatment.

All male goldfish were strippable when goldfish pituitary was administered, but only 66.6 per cent of the males reached the strippable stage with Bonneville cisco pituitary treatment (Table 12 and Figure 11). When using brown trout pituitary injections, only 16.7 per cent of the male goldfish reached the intermediate stage and none were strippable. The pituitaries from mountain whitefish and largemouth bass gave no response. The time of pituitary collection from various donors is given under material and methods on page 4.

It was observed that the effects of goldfish pituitary injection to

Table 11. Response of male goldfish when treated with injected and uninjected goldfish pituitary at 1.83 mg%.

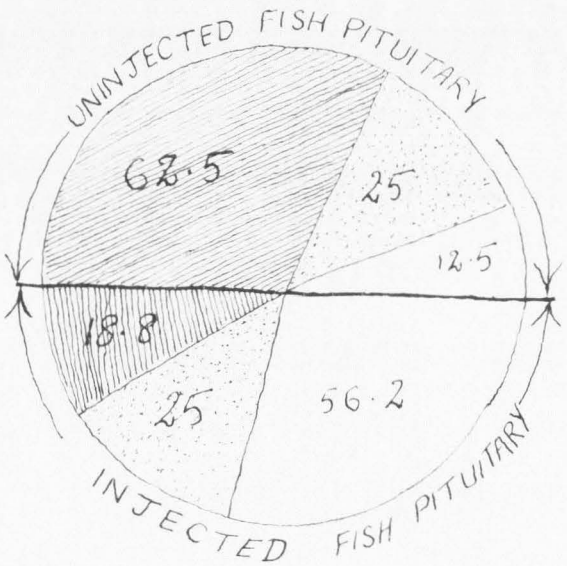
Treatment (source of pituitary)	Response in percentage			Total percentage	
	Strippable	Intermediate	Hard	Affected	Not affected
Injected fish pituitary	18.8	25	56.2	43.8	56.2
Uninjected fish pituitary	62.5	25	12.5	87.5	12.5

Fish were processed 16 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Injected fish pituitary vs. Uninjected fish pituitary	1	8.8	.995

[1]The fraction of male goldfish affected when injected with mature-ripe and mature fish pituitary are significantly different with a probability of .995.



Sample size: 32 goldfish, 16 for each treatment

Strippable

Intermediate

Hard

Figure 10. Response (in percentage) of male goldfish when treated with injected and uninjected goldfish pituitaries at 1.83 mg%. Fish were processed 16 hours after injection.

Table 12. Response of male goldfish when treated with pituitaries from different donors each at 1.83 mg%.

Donors	Response in percentage			Total percentage	
	Strippable	Intermediate	Hard	Affected	Not affected
Cisco	66.6	16.7	16.7	83.3	16.7
Brown trout	--	16.7	83.3	16.7	83.3
Mountain whitefish	--	--	100	--	100
Largemouth bass	--	--	100	--	100
Goldfish	100	--	--	100	--

Fish were processed 12 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	4	22.1	.995
Goldfish pituitary vs. rest	1	11.4	.995
Cisco pituitary vs. rest	1	3.5	.90
Brown trout pituitary vs. rest	1	3.4	.90

[1]Goldfish pituitary injection is significant with a probability of .995. Cisco and brown trout pituitary injections are significantly different with a lower probability of .90.

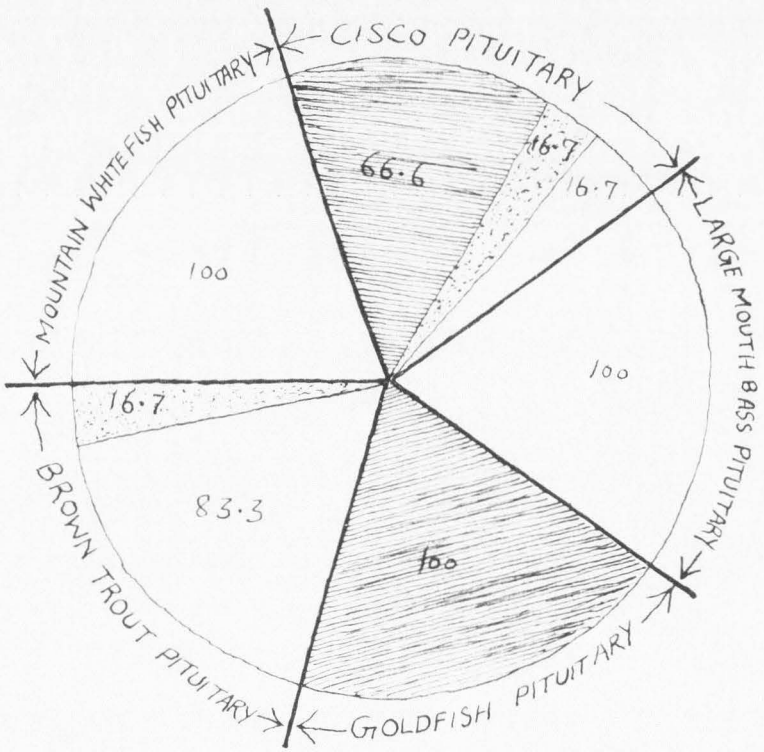


Figure 11. Response (in percentage) of male goldfish when treated with pituitaries from different donors each at 1.83 mg%. Fish were processed 12 hours after injection.

goldfish were highly significant. Goldfish pituitary to goldfish seem to be even more effective than carp pituitary to goldfish. This may point to species specificity. Pituitary injections from largemouth bass, which has a taxonomically remote relation to goldfish, had no effect although the pituitaries were collected from ripe fish just before the breeding season. It seems, therefore, that the nearer the relation of recipient and donor, the better is the response.

Pituitaries collected from fish before their breeding period are much more effective than those collected after the breeding season. Carp pituitary injections used in other experiments show this significant difference. Although brown trout, mountain whitefish, and Bonneville cisco have a close relation to each other, they showed different effects. Cisco pituitaries collected before their breeding season showed good response in goldfish. Mountain whitefish pituitary injections were not effective and brown trout showed very little effect, probably because their pituitaries were collected after they had spawned.

The first recognition that specificity in gonadotropins might exist came from Pickford and Atz (1957) who pointed out that female Bufo arenarum did not respond to implants of pituitaries from animals belonging to other classes, including one species of fish. Such differences appreciably affect the efficiency of biological activity of the gonadotropins when administered to animals of widely divergent taxonomic relationship from the hormonal donor.

Effects of intraperitoneal and intramuscular injections of pituitary on goldfish.--Male goldfish were selected as the experimental fish for this study. They were given pituitary injections of 1.83 mg% of carp pituitary. These pituitaries were collected before the carp had spawned.

Intraperitoneal injections were given in the body cavity near the pelvic fin. Intramuscular injections were made in the muscle on the caudal peduncle. Fish were processed 10 hours after treatment.

Of the males, 93.1 per cent were fully strippable, and 6.9 per cent were at the intermediate stage when given intraperitoneal injections of carp pituitary (Table 13, Figure 12). Of goldfish that were given intramuscular injections, only 51.8 per cent of the males were strippable, 20.7 per cent reached the intermediate stage, and 27.5 per cent were not affected.

Study on influences of environmental factors on fish administered pituitary hormones

Effects of gravel and vegetation on goldfish after pituitary injection.--For this study, gravel was placed in one set of the bottles; in another set, both gravel and vegetation were used; while in the third, neither was used. The gravel was a mixture of thoroughly washed fine stones. Only floating plants, Myriophyllum sp., were used as vegetation. Male goldfish were the experimental fish and were injected at 1.83 mg% with carp pituitary. The carp pituitaries were collected in July 1960 after 50 per cent of the carp had spawned. The fish were processed 16 hours after treatment.

Some 68.2 per cent of the males were affected in bottles with gravel and vegetation (Table 14, Figure 13). The same percentage of fish were affected in bottles without gravel and vegetation. In bottles with gravel only, 72.7 per cent of the males were affected. This percentage increase of affected fish in bottles with gravel is, however, not significantly different.

In another experiment it was observed that rooted vegetation

Table 13. Response of male goldfish to intraperitoneal and intramuscular injections, each at 1.83 mg% of carp pituitary.

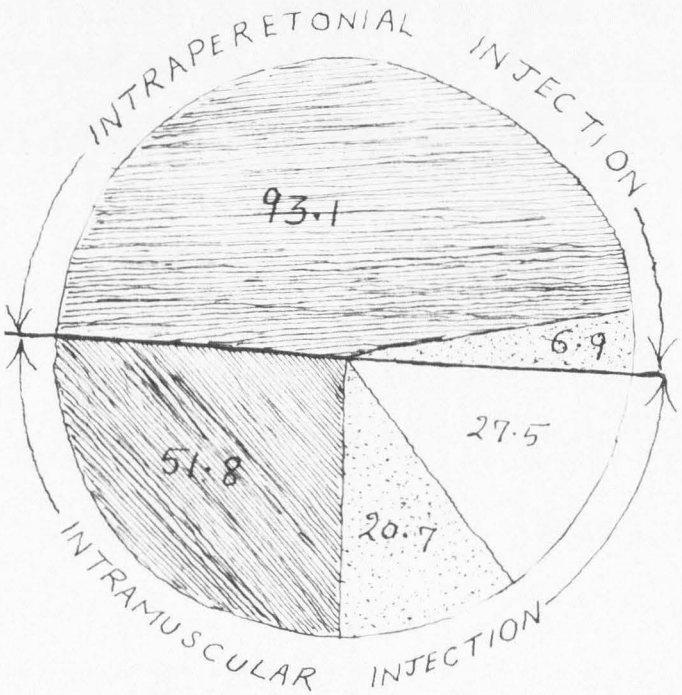
Treatment	Type of injection	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	I.P.	93.1	6.9	--	100	--
	I.M.	51.8	20.7	27.5	72.5	27.5
Control	I.P.	--	--	100	--	100
	I.M.	--	--	100	--	100

Fish were processed 10 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	3	38.2	.995
Treatment vs. control	1	28.2	.995
I.P. vs. I.M.	1	7.1	.99

[1]The fraction of goldfish affected when given intraperitoneal and intramuscular injections of carp pituitary are significantly different with a probability of .99.



Sample size: 58 goldfish, 29 for each treatment

Strippable
Intermediate
Hard

Figure 12. Response (in percentage) of male goldfish to intraperitoneal and intramuscular injections of carp pituitary at 1.83 mg%. Fish were processed 10 hours after treatment.

Table 14. Effects of gravel and vegetation on male goldfish after carp pituitary injection at 1.83 mg%.

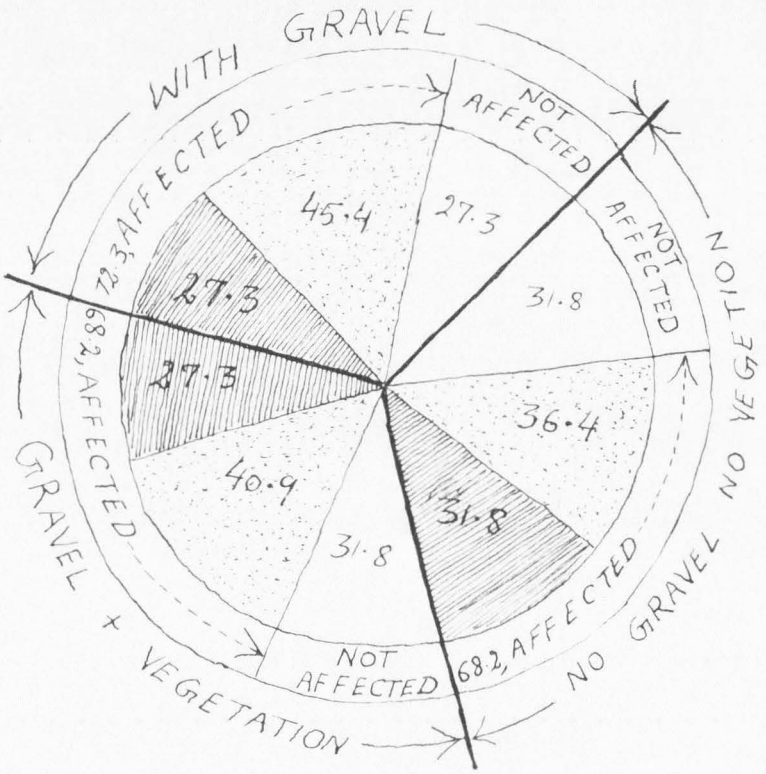
Treatment	Type of environment	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	With gravel	27.3	45.4	27.3	72.7	27.3
	Gravel and vegetation	27.3	40.9	31.8	68.2	31.8
Control	No gravel No vegetation	31.8	36.4	31.8	68.2	31.8

Fish were processed 16 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	2	.11	.05
Control vs. rest	1	.08	.1

[1] Effect of gravel and vegetation on fish injected with pituitary is not significant within 16 hours after injection.



Sample size: 66 fish, 22 for each treatment

Strippable

Intermediate

Hard

Figure 13. Effects (in percentage) of gravel and vegetation on male goldfish after carp pituitary injections at 1.83 mg%. Fish were processed 16 hours after treatment.

(Vallisneria sp.) had a greater effect on ripening of goldfish than the floating vegetation. In this test male goldfish were used. Vallisneria sp. was placed in one of the aquaria, Myriophyllum sp. in the second, and no vegetation in the third. The Vallisneria sp. plants were kept in the rooted position by a weight. Only four male goldfish were put in each of the aquaria after injection with carp pituitary at the 0.22 mg% level. The fish were processed 60 hours after treatment. It was observed that in aquaria with Vallisneria sp. all four goldfish reached the intermediate stage. In the aquaria with Myriophyllum sp. two of the males were at the intermediate stage and two were in the hard condition. In the aquaria without vegetation, there was one male that had reached the intermediate stage, but the other three were not affected.

In another experiment the effect of gravel and gravel-vegetation was observed. Male goldfish were used as experimental fish. They were given injections of carp pituitary at the 0.22 mg% level. The fish were processed 60 hours after treatment. Of the four fish in the aquarium with gravel only, two fish were at the intermediate stage and two were not affected. In the aquarium with gravel and vegetation (Vallisneria sp.) one male was fully strippable and three were at the intermediate stage. In the aquarium without gravel or vegetation, there was one male which had reached the intermediate stage, but the other three were not affected.

Effect of temporary partition on male and female goldfish after pituitary injection.--This experiment was conducted in aquaria which were partitioned with glass slabs. Male and female goldfish in the ratio of 1:1 were released. Control fish were given equal treatment except that the aquaria were not partitioned. Male and female goldfish were injected with 1.83 and 4.16 mg% of goldfish pituitary respectively. Pituitaries used in

this experiment were collected in the laboratory from treated fish used in other experiments. Male and female goldfish pituitaries were mixed in almost equal numbers. This collection included pituitaries from hard and control fish. Pituitaries from intermediate and strippable fish were not mixed in this collection. Fish were examined 8 hours after injection (Table 15, Figure 14).

It was observed that in the partitioned aquaria males were continuously brushing against the transparent glass. Males were continually trying to go to the other side with the females. The partition was removed 3 hours after injection. The males, immediately after release, seemed to be very much excited and were constantly chasing the females. The females avoided the males for about an hour after their release. Later the females were equally responsive. Males in the unpartitioned aquaria also chased the females, but their chase was often irregular. Males in the unpartitioned aquaria were not as responsive as they were in partitioned aquaria. Unlike females in the partitioned aquaria which were equally responsive 1 hour after the injections, females in the unpartitioned aquaria never reached this stage. On processing 8 hours after the injection, it was observed that the percentage of ripe females in the aquaria with temporary partitions was 44.4 as compared to none in the unpartitioned aquaria. It seems that temporary partition does have some psychological effect which makes the males more excited. This constant chase, loveplay, and courtship of females by males seemed to enhance earlier ripening of females.

Effect of sex ratio on response of goldfish after pituitary injection. --Male and female goldfish were partitioned for the first 3 hours after injection. A partition was made by inserting a glass slab in the aquaria.

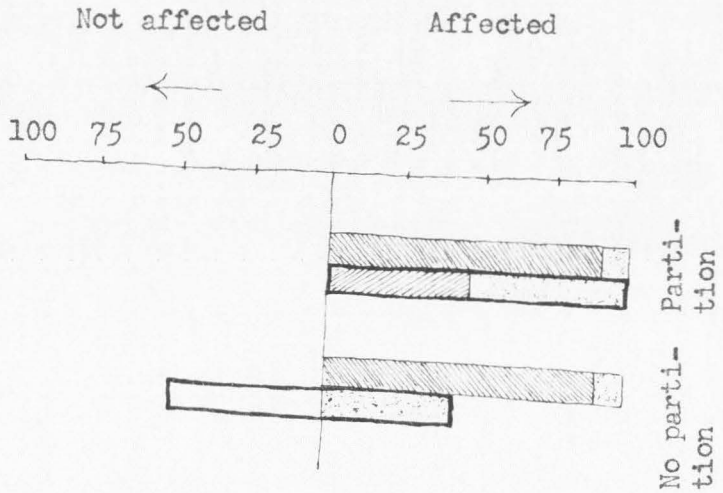
Table 15. Effect of temporary partition on male and female goldfish after goldfish pituitary (mature) injection at 1.83 and 4.16 mg% respectively.

Treatment	Sex	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Partition	♀	44.4	55.6	--	100	--
	♂	88.9	11.1	--	100	--
No partition	♀	--	55.6	44.4	55.6	44.4
	♂	88.9	11.1	--	100	--

Fish were processed 8 hours after treatment.

	Statistical Analysis[1]		
Source of variation	d/f	X^2	Probability
Among treatments	1	2.5	.10
Males (partition) vs. male (no partition)	1	0	--
Female (partition) vs. female (no partition)	1	2.9	.90

[1]Effect of partition on male goldfish after pituitary injection is not significant statistically. There is, however, some difference in strippable fish between partitioned and unpartitioned female goldfish. The fraction of fish within females (partitioned versus no partition) is significant with a probability of .90.



Sample size: 36 goldfish, 18 for each treatment (9 ♂ and 9 ♀ in each treatment)

Males
Females
Strippable
Intermediate
Hard

Figure 14. Effect of temporary partition on male and female goldfish after goldfish pituitary injection at 1.83 mg% and 4.16 mg%, respectively. Fish were processed 8 hours after treatment.

Males and females were put in at the ratio of 1:1, 1:2, 1:3, 1:4; males on one side and females on the other. Male and female goldfish were injected with 1.83 and 4.16 mg% of goldfish pituitary respectively. These pituitaries were collected in the laboratory from previously treated fish. Pituitaries from males and females were mixed in almost equal numbers. Only pituitaries from control fish and those which remained in the hard condition were included. The fish were processed 24 hours after treatment.

Males and females in the ratio of 1:1 and 1:2 were affected (Table 16, Figure 15). In the 1:3 ratio 44.4 per cent of the females were not affected. Some 75 per cent of the 1:4 ratio females were not affected. The partition, however, had no effect on the males.

Effect of handling on goldfish after pituitary injection.--One group of fish was handled every hour after treatment with pituitary hormone, the second group of fish was handled after every 2 hours, and the third group after every 6 hours. The fourth group of fish was handled only once at the time of processing. Fish were processed 12 hours after injection. Thus fish were handled 12 times, 6 times, twice, and once respectively in 12 hours time after injection. In this experiment only male goldfish were used as experimental fish. All fish were given injections of carp pituitary at the 1.83 mg% level.

In the group of fish handled every hour, only 36.3 per cent of the males were affected (Table 17, Figure 16). There was a steady increase of strippable fish as handling decreased. The group of fish that was handled only once at the time of processing had 85.8 per cent of the fish affected.

Table 16. Effect of sex ratio on response of goldfish after goldfish pituitary injections to males and females at 1.83 mg% and 4.16 mg%, respectively.

Ratio of male to female	Sex	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
1:1	♂	100	--	--	100	--
	♀	100	--	--	100	--
1:2	♂	100	--	--	100	--
	♀	66.7	33.3	--	100	--
1:3	♂	100	--	--	100	--
	♀	--	56.6	44.4	56.6	44.4
1:4	♂	100	--	--	100	--
	♀	--	25	75	25	75

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	3	12.6	.995
1:1 and 1:2 vs. 1:3 and 1:4	1	8.3	.995
Male 1:1 vs. male 1:4	1	0	--
Female 1:1 vs. female 1:4	1	2.9	.90

[1]There is no difference in the fraction of male goldfish affected whether the ratio of male and female is 1:1 or 1:4. However, ratio of male to female had influence on ripening of female goldfish. Response of females is significantly different with a probability of .90 when the ratio is changed from 1:1 to 1:4.

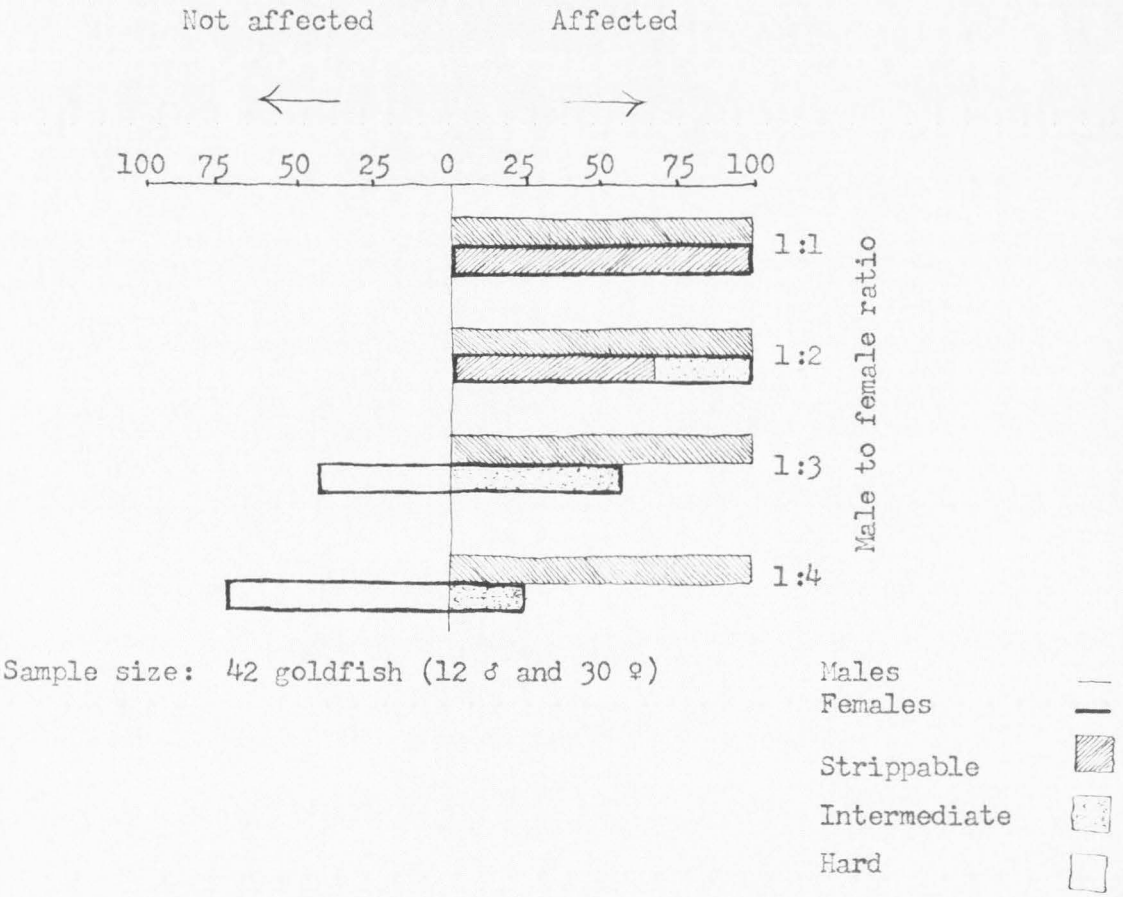


Figure 15. Effect of sex ratio on response of goldfish after goldfish pituitary injection at 1.83 mg% and 4.16 mg% to males and females, respectively. Fish were processed 24 hours after treatment.

Table 17. Effect of handling on response of male goldfish after carp pituitary injection at 1.83 mg%.

Treatment	Handling after every	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
	1 hour	9.1	27.2	63.7	36.3	63.7
	2 hours	14.2	47.6	38.2	61.8	38.2
	6 hours	31.8	54.7	13.6	86.4	13.6
Control	No handling	42.9	42.9	14.2	85.8	14.2

Fish were processed 12 hours after treatment.

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	3	16.7	.995
Treatment vs. control	1	5.3	.975
1 hour vs. no handling	1	13.1	.995

[1]The difference between goldfish that were handled every hour and those that were not handled during the treatment is significant with a probability of .995.

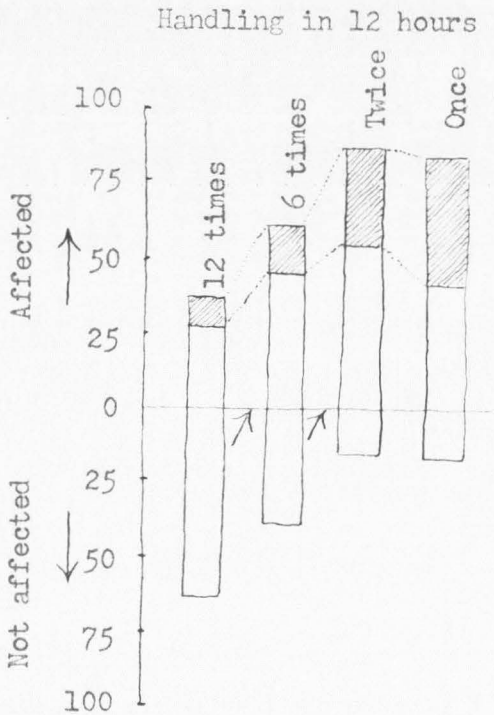


Figure 16. Effect of handling on response of male goldfish after carp pituitary injection at 1.83 mg%. Fish were processed 12 hours after treatment.

Effect of prestarvation and feeding of goldfish before pituitary injection.--For this experiment, one group of fish was starved for 7 days, a second for 5 days, a third for 3 days, and a fourth for 1 day only. Food was given to a fifth group of fish just 1 hour before the experiment. Male goldfish were used as the experimental fish. They were given injections of carp pituitary at the 1.83 mg% level. The carp pituitaries were collected in July 1960 when 50 per cent of the carp had spawned. Fish were processed 16 hours after treatment.

Some 25 per cent and 18.7 per cent of the treated fish died among the group of fish starved for 7 days and 5 days respectively (Table 18, Figure 17). There was no mortality of the fish starved for 3 days and 1 day. In the group of fish to which food was given before the experiment, 31.3 per cent died. The percentage of strippable fish in the group starved for 1 day was maximum. Overstarvation had an adverse effect on the ripening of fish.

Effect of temperature on response of goldfish after pituitary injection.--No suitable arrangement for controlled temperature experiments could be made. It was noticed, however, that there was a difference in temperature of the water between the experimental bottles kept on the table and those placed on the floor. The water in the bottles placed on the floor was 3° F. higher than in the bottles on the table. The average temperature of the water in the bottles kept on the floor was 78° F. and those on the table were 75° F. Male goldfish were used as the experimental fish in this study. Fish were given carp pituitary injections of 1.83 mg%. These carp pituitaries were collected in July 1960 and 50 per cent of the carp had spawned. The experimental fish were processed 16 hours after treatment.

Twenty-two per cent of the fish were strippable in the bottles kept on the table as compared to 50 per cent strippable fish in the bottles placed

Table 18. Effect on response of male goldfish after prestarvation and feeding just before pituitary injection at 1.83 mg% of carp pituitary.

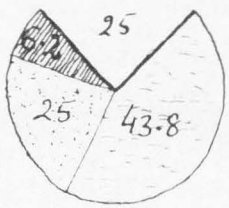
Treatment	Time of starvation	Response in percentage			Total percentage		
		Strippable	Intermediate	Hard	Affected	Not affected	% died
	7 days	6.2	25	43.8	31.2	43.8	25
	5 days	12.5	25	43.8	37.5	43.8	18.7
	3 days	31.2	37.6	31.2	68.8	31.2	--
	1 day	50	18.8	31.2	68.8	31.2	--
Control	Feeding	18.7	43.8	6.2	62.5	6.2	31.3

Fish were processed 16 hours after treatment.

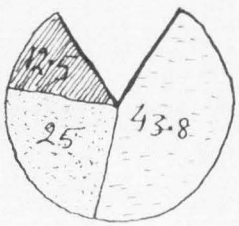
Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	4	8.0	.90
Treatment vs. control	1	5.8	.975
3-day and 1-day vs. rest	1	.40	.10
1-day vs. feeding	1	.79	.10

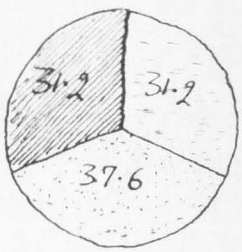
[1]The fraction of starved goldfish affected compared to the fish which were fed is significantly different with a probability of .975.



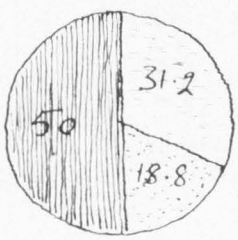
Starved for 7 days



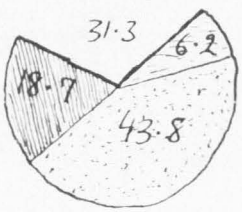
Starved for 5 days



Starved for 3 days



Starved for 1 day



Feeding

Sample size: 80 goldfish, 10 for each treatment

Strippable
Intermediate
Hard
Died

Figure 17. Effect (in percentage) on response of male goldfish after prestarvation and feeding just before the pituitary injection at 1.83 mg% of carp pituitary. Fish were processed 16 hours after treatment.

on the floor (Table 19, Figure 18).

Burger (1939) found that in Fundulus sp. spermatogonial multiplications took place at a temperature of about 50° F., but that spermatogenesis did not proceed further until higher temperatures were reached. Bullough (1939) determined that higher temperatures (about 63° F.) accelerated the formation of primary spermatocytes or the early part of the secondary growth phase of the oocytes in Phoxius sp. in winter, but further development depended on the addition of extra illumination. Under these conditions spermatozoa were formed and eggs grew considerably in size, although they did not mature. In contrast, fish subjected to low temperatures (about 45° F.) and longer illumination showed no gonadal change. It seems evident that under the conditions of Bullough's experiments, high temperature alone was not able to bring about the production or release of sufficient gonadotropin to carry along gametogenesis.

Study on preservation of fish pituitary glands

Effect of temperature on hormone activity of excised pituitary glands. --Pituitaries from treated male and female goldfish were mixed in about equal numbers. This collection included pituitaries from intermediate and strippable fish only; pituitaries from control fish and fish in the hard condition were not included. One set of these pituitaries was kept in a dark vial inside a desiccator in the refrigerator at 40° F. The other set of pituitaries was kept in a dark vial in a desiccator at room temperature at 75° F. Both sets of pituitaries were kept for 3 days. Female goldfish were used as experimental fish. These two sets of pituitaries were injected into two groups of females, each at the 4.16 mg% level. The fish were processed 16 hours after treatment.

The per cent of females which were fully strippable after injection of

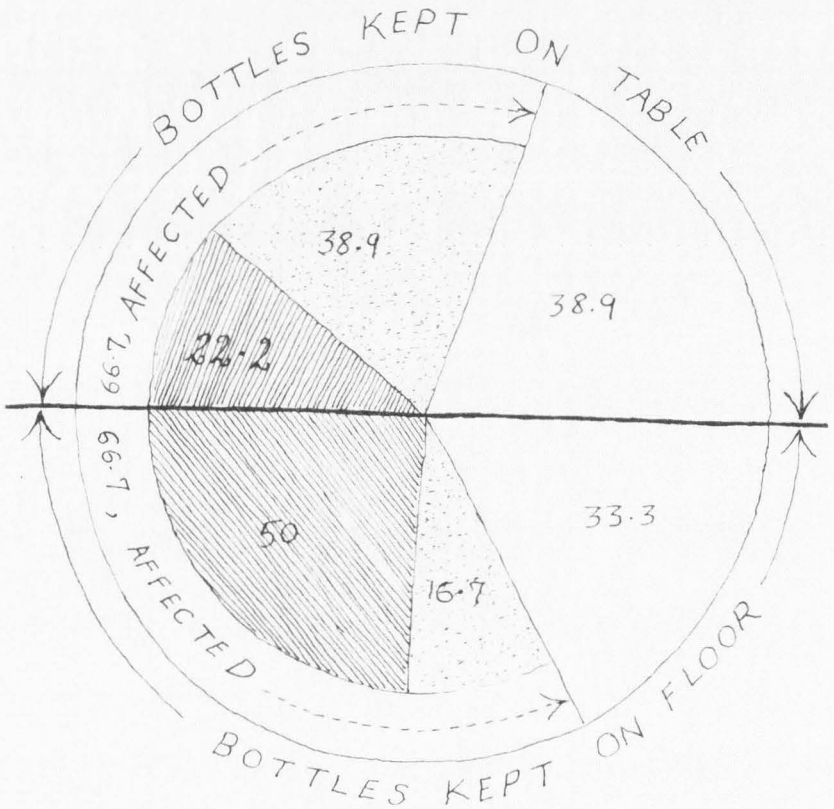
Table 19. Effect of 3° F. temperature difference on response of male goldfish after carp pituitary injection at 1.83 mg% (temperature in the bottles placed on the floor was 3° F. higher than in the bottles placed on the table).

Treatment	Bottles placed on	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
Treated fish	Table	22.2	38.9	38.9	61.1	38.9
	Floor	50	16.7	38.3	66.7	33.3
Control $D.H_2O$	Table	--	--	100	--	100
	Floor	--	--	100	--	100

Fish were processed 16 hours after treatment.

	Statistical Analysis[1]		
Source of variation	d/f	X^2	Probability
Among treatments	3	8.5	.95
Treatment vs. control	1	6.09	.975
On table vs. on floor (treated)	1	.48	.10

[1]Effect of 3° F. temperature difference between bottles on table and floor is not significant statistically. However, there is some difference in strippable fish as discussed under the experiment.



Sample size: 36 goldfish, 18 for each treatment

Average temperature of the bottles on the table and the floor was 75° F and 78° F respectively.

Strippable

Intermediate

Hard

Figure 18. Effect (in percentage) of 3° F. temperature difference on response of male goldfish after carp pituitary injection at 1.83 mg%. Fish were processed 16 hours after treatment.

the pituitaries which had been kept in the refrigerator was 41.7 (Table 20, Figure 19). In the group of females injected with the pituitary kept at room temperature there were no fish which reached the fully strippable stage.

Effect of light on hormone activity of excised pituitary glands.--In this study the pituitaries of treated goldfish used in other experiments were utilized. Pituitaries from male and female goldfish were mixed in equal numbers. This collection included pituitaries from intermediates and fish that were fully strippable only; pituitaries from control fish and fish in the hard condition were not used. A batch of these pituitaries was kept in a dark vial in a desiccator inside a refrigerator. The other batch of pituitaries was kept in a transparent vial which was placed in the desiccator beside the pituitaries in the dark vial. These two sets of pituitaries, in the dark vial and in the transparent vial, were kept in a refrigerator for 13 days. The door of the refrigerator was opened on an average of twice a day for 1 to 2 minutes for 13 days in order to remove things from the refrigerator. Thus the pituitaries in transparent vials were exposed to light during this period. Female goldfish were used as experimental fish. These two batches of pituitaries were injected into two groups of females at the 4.16 mg% level. The fish were processed 16 hours after treatment. Some 25 per cent of the females reached the fully strippable stage in the group of fish which were given injections from the pituitaries kept in the dark vial, but none reached this stage in the group of females which were injected with the pituitaries kept in the transparent vial (Table 21, Figure 20).

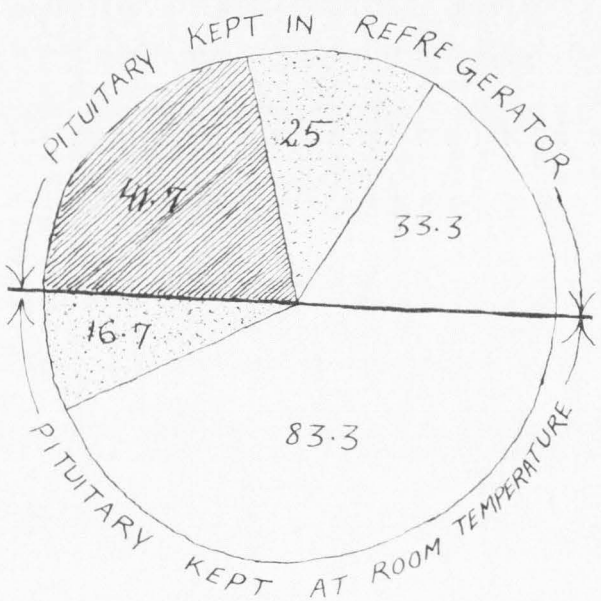
Effect of fresh and preserved pituitaries on goldfish.--Pituitaries were collected from previously treated female goldfish which had well

Table 20. Effect of hormone activity of excised pituitary glands kept at room temperature (about 75° F.) and in the refrigerator (about 40° F.).

Pituitary kept	Response in percentage			Total percentage	
	Strippable	Intermediate	Hard	Affected	Not affected
In refrigerator	41.7	25	33.3	66.7	33.3
At room temperature	--	16.7	83.3	16.7	83.3

	Statistical Analysis[1]		
Source of variation	d/f	X^2	Probability
In refrigerator vs. at room temperature	1	4.28	.95

[1]The difference between goldfish injected with refrigerated pituitary and those injected with nonrefrigerated pituitary is significant with a probability of .95.



Sample size: 24 female goldfish, 12 for each treatment

Each was injected with 4.16 mg% of goldfish pituitary. The fish were processed 16 hours after injection.

Strippable

Intermediate

Hard

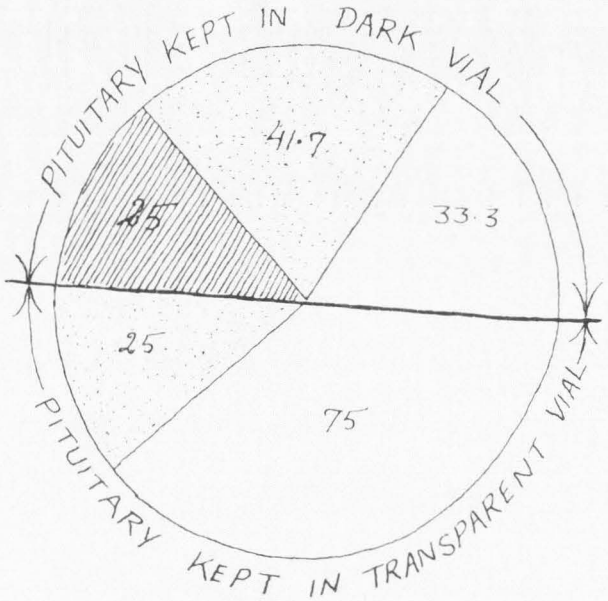
Figure 19. Effect (in percentage) on hormone activity of excised pituitary glands kept at room temperature (about 75° F.) and in the refrigerator (about 40° F.).

Table 21. Effect on hormone activity of excised pituitary glands kept in transparent and dark vials.

Pituitary kept in	Response in percentage			Total percentage	
	Strippable	Intermediate	Hard	Affected	Not affected
Dark vial	25	41.7	33.3	66.7	33.3
Transparent vial	--	25	75	25	75

	Statistical Analysis[1]		
Source of variation	d/f	X^2	Probability
Dark vial vs. transparent vial	1	2.7	.90

[1]Differences between goldfish injected with pituitary kept in dark vials and in transparent vials are significant statistically with a probability of .90.



Sample size: 24 female goldfish, 12 for each treatment

Each was injected with 4.16 mg% of goldfish pituitary and processed 16 hours after treatment.

Strippable

Intermediate

Hard

Figure 20. Effect in percentage on hormone activity of excised pituitary glands kept in the transparent and dark vials.

developed gonads. This collection included pituitaries from control fish and from fish which were in a hard condition. Male goldfish were used as experimental fish and were injected at the 0.45 mg% level. The fish were processed 16 hours after injection.

In one experiment the effect of fresh pituitaries and pituitaries preserved in alcohol were compared. Pituitaries were kept in alcohol for 15 days. There were 79.2 per cent strippable fish in the group of fish injected with fresh pituitaries (Table 22, Figure 21); and of the fish that were given injections from alcohol preserved pituitaries, 72.8 per cent reached this stage.

In another experiment the effects of alcohol and acetone preservation were compared. Pituitaries were preserved in alcohol and acetone for 6 days. Some 88 per cent of the fish in the group injected with pituitaries preserved in acetone were fully strippable; while among those injected with pituitaries preserved in alcohol, 75 per cent reached this stage (Table 23, Figure 22).

Desiccation of pituitary glands with acetone was used by Houssay and Brasotte (1931) (Pickford and Atz, 1957). In the U.S.S.R., this method has been found so satisfactory that practically no attempt to improve upon it has ever been made. Von Ihering (1935) tested acetone as a preservative and compared it with other methods of preservation. Ball and Bacon (1954) noted no gross difference in strength between fresh frozen carp pituitaries and those treated with cold acetone for 36 hours, then dried on filter paper and stored under refrigeration. The Brazilians, however, have had much more success with absolute alcohol as a preservative. In fact, this is now the standard preserving agent used by nearly all their pisciculturists (Fontenele, 1955).

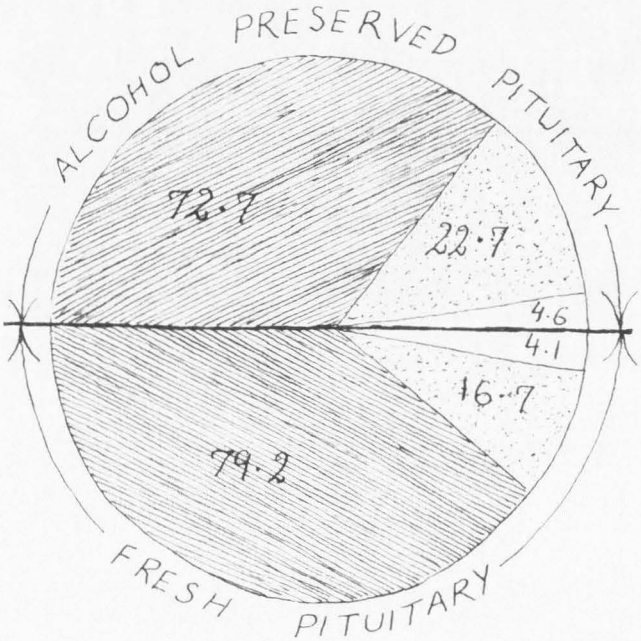
Table 22. Effects of hormones of fresh pituitary glands and those preserved in alcohol.

Treatment	Preservative	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
	Alcohol	72.7	22.7	4.6	95.4	4.6
	Fresh	79.2	16.7	41	95.9	4.1
Control $D.H_2O$		--	--	100	--	100

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	2	50.2	.995
Treatment vs. control	1	45.4	.995
Alcohol vs. fresh	1	.61	.10

[1]Difference between fish treated with pituitary implants and control fish injected with distilled water is significant. Injections of alcohol preserved and fresh pituitary are, however, not significantly different.



Sample size: 46 male goldfish

Each was injected with 0.45 mg% of goldfish pituitary and processed 16 hours after treatment.

Strippable

Intermediate

Hard

Figure 21. Effect (in percentage) of alcohol preservation on hormone activity of excised pituitary glands.

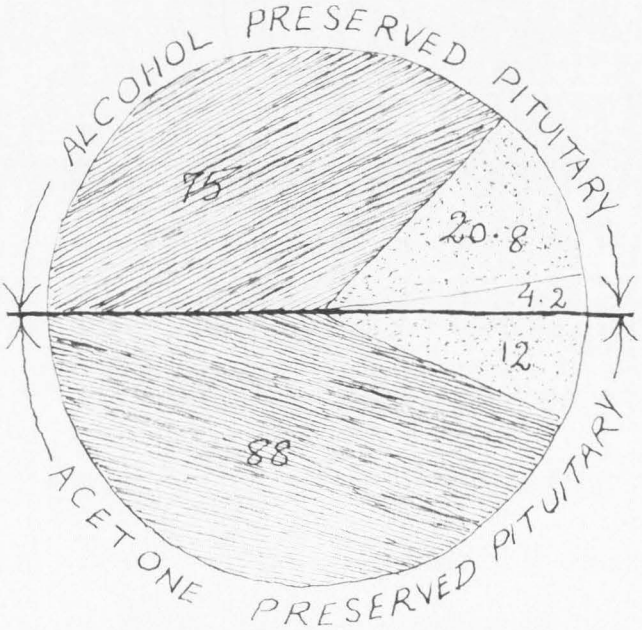
Table 23. Effect of alcohol and acetone preservation on hormone activity of excised pituitary glands.

Treatment	Preservative	Response in percentage			Total percentage	
		Strippable	Intermediate	Hard	Affected	Not affected
	Alcohol	75	20.8	4.2	95.8	4.2
	Acetone	88	12	--	100	--
Control $D.H_2O$		--	--	100	0	100

Statistical Analysis[1]

Source of variation	d/f	X^2	Probability
Among treatments	2	62.2	.995
Treatment vs. control	1	57.4	.995
Alcohol vs. acetone	1	4.16	.95

[1]Effect of alcohol and acetone preserved pituitaries is significantly different with a probability of .95.



Sample size: 50 male goldfish

Each was injected with 0.45 mg% of goldfish pituitary and processed 16 hours after treatment.

Strippable

Intermediate

Hard

Figure 22. Effect (in percentage) of alcohol and acetone preservation on hormone activity of excised pituitary glands.

Spawning reflex and behavior of fish after pituitary injection

These observations were made in an aquarium after pituitary hormone injection. Immediately after injection the fish were often inactive and motionless although opercular movement had increased manyfold. After 2 to 3 hours the males were darting about rapidly from one corner of the aquaria to the other, but the females were still inactive. Not until about 4 hours after the injection did the fish show an interest in one another. After about 5 to 6 hours the males generally stopped darting. They then seemed to become conscious of the presence of the females. After this males would chase females for a while and then stop suddenly followed by an interval of aimless darting. After 7 to 8 hours, males almost continuously chased females. In certain cases, when all females were removed from the aquaria, males often leaped out of the water and injured themselves. Females always avoided males until about 8 to 9 hours after injection. After this females did not swim away from males. At this time, both males and females seemed to be equally responsive. The male then circled the female, sometimes nibbling about the vent which hung obliquely down below the female. Sometimes the males gave a sort of soft biting on the cheeks and often rubbed their bodies against the females. Soon females also started nibbling and giving a soft bite in response to males. Both became excited and began exhibiting fast undulation of the caudal fin swinging in perfect harmony. There was a distinct, rapid, and violent vibration of their fins. Parallel to each other, males often pushed females and squeezed them against the wall of the aquarium. A peak of activity was often reached in 10 to 12 hours after injection and lasted for 2 to 3 hours. After this there seemed to be a slow decline of response. In some cases, especially in males, there was a very abrupt fall of response, and

they were seen going away from females.

On examination, the vent looked somewhat reddish and swollen. Both males and females had a very pulpy body just behind and dorsal to the pelvic fin. With a slight touch, eggs or milt oozed out in a considerable amount. They did not spawn in the aquaria, probably due to a lack of certain environmental conditions.

SUMMARY AND CONCLUSIONS

There is an increasing demand for quality fish spawn for fish culture development. Also, the construction of more and more dams has resulted in insurmountable obstacles for ascending and descending fish which may ultimately seriously reduce some fisheries. Thus, some measure of artificial propagation should be taken to safeguard valuable fishery resources. A partial solution of supplementing natural propagation is accomplished by inducing fish to spawn artificially in the hatchery. A method of doing this is by stimulating fish to spawn by the use of pituitary hormones.

In this study goldfish were used as experimental fish, and various experiments on pituitary injections were conducted. The influence of environmental factors on fish administered with pituitary hormones is evaluated. Some study on preservation of pituitary glands was made. Spawning reflex and behavior of goldfish after the pituitary hormone injections were observed. The following conclusions were drawn from this study:

1. Male goldfish reached the full strippable stage within 12 to 18 hours after the injection of carp pituitary at 1.83 mg%.

2. Female goldfish required much higher doses than males and reached the full strippable stage within 12 to 18 hours after injection of carp pituitary at 4.16 mg%.

3. Female goldfish when administered a small dose of 1.83 mg% of

carp pituitary showed some indications of the effect of hormones 30 hours after injection.

4. Carp pituitary injections in a series of small doses had much better effect on ripening of both male and female goldfish than the same amount in one massive dose. The fish not only reached the ripe stage earlier but also required a lower concentration of pituitary when they were given injections in a series of doses.

5. Female goldfish pituitary is more effective than male goldfish pituitary.

6. Mature goldfish pituitary injected into goldfish is much more effective than immature goldfish pituitary.

7. Pituitary from injected goldfish is less effective than uninjected goldfish pituitary.

8. Pituitaries collected from mature carp, brown trout, and mountain whitefish after they had spawned were less effective than pituitaries collected from mature carp and Bonneville cisco before spawning.

9. Pituitary injection is probably more effective if the taxonomic relationship of the donor and recipient is close; for example, goldfish to goldfish or carp to goldfish rather than more remote relations such as largemouth bass to goldfish.

10. Intraperitoneal injection of carp pituitary was more effective than intramuscular injection of carp pituitary.

11. Gravel and vegetation had a negligible effect over a 16-hour period on goldfish injected with pituitary glands. However, gravel and vegetation had some influence on ripening of goldfish when the duration of the experiment was increased to 60 hours or more after treatment. Rooted vegetation like Vallisneria sp. had a greater effect on early

ripening of goldfish than floating vegetation like Myriophyllum sp.

12. Temporary separation of male and female goldfish after pituitary treatment has some psychological effect. This separation particularly excited males and induced them to indulge in loveplay and courtship after they were released. The excitement of males seemed to enhance the earlier ripening of females. The glass partition, however, had no effect on ripeness of males.

13. A suitable ratio of male to female goldfish seems to be 1:1 or 1:2. Further increase of females resulted in reduction of ripeness among females. An increase of females up to 1:4, however, did not affect ripeness of the males.

14. Handling of goldfish treated with pituitary hormones had a strikingly adverse affect on ripeness of the fish.

15. It was observed that starving goldfish for 1 to 2 days prior to pituitary hormone treatment had a beneficial effect on ripeness of fish. Feeding before the treatment or overstarvation resulted in death of many experimental fish.

16. Temperature had great influence on early ripening of goldfish treated with pituitary hormones. So small a temperature change as 3° F. increased the number of strippable fish after the pituitary treatment.

17. Exposure to temperature and light decreased the effectiveness of hormone-containing pituitary glands.

18. Storing vials containing pituitary glands outside the desiccator did not decrease the effect of pituitary hormones as long as the vial was tightly closed.

19. There was almost no difference between the effects of fresh and alcohol preserved pituitaries within 6 days time. Acetone appeared to be

a better preservative than alcohol.

20. Male goldfish exhibited a spawning reflex much earlier than females. In the beginning males and females were observed behaving independently of each other. Ten to 12 hours after pituitary hormone treatment, however, both sexes were found to be equally responsive.

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